

In the Specification

Please amend the Brief Description of Drawings paragraph beginning on page 4, line 18:

**Figures 1A-1D** show FIV Western blot analysis of subjects #FH1 and #FH2. FIV<sub>Shi</sub> (D) and FIV<sub>Bang</sub> (B) Western blots (Figures 1A-1C) were reacted with sera from subjects #FH1, #FH2, and #FH5 (control individual with minimum cat exposure) for 20 hours. Experimentally FIV-infected cat (Cat +) was used as the source of strongly reactive control serum and uninfected SPF cat (Cat -) was used as the source of non-reactive control serum. Key bands are highlighted with an arrowhead on the left. Figure 1D: Virus neutralizing antibodies to FIV and HIV were detected in cultures. a Western blot of human sera on FIV<sub>Shi</sub>.

**~~Figures 2A and 2B~~ Figures 2A-2F** show alignment of gag sequences of cat #FC1 and subject #FH1. ~~Figure 2A shows~~ Figures 2A-2D show alignment of gag nucleotide sequences. Figure 2B shows Figures 2E-2F show alignment of gag amino acid sequences. Gag sequences of the nine clones isolated from cat #FC1 and subject #FH1 are shown in comparison to the consensus sequence of cat #FC1 (top sequence). Hyphens denote nucleotide or amino acids identical to the consensus sequence derived from cat #FC1 and those, which differ from the consensus, are presented with the appropriate nucleotide or amino acid symbols. In Figures 2A-2D the nucleotide consensus sequence is SEQ ID NO: 3; FC1 #4 is SEQ ID NO: 4; FC1 #5 is SEQ ID NO: 5; FC1 #6 is SEQ ID NO: 6; FC1 #10 is SEQ ID NO: 7; FC1 #12 is SEQ ID NO: 8; FC1 #13 is SEQ ID NO: 9; FC1 #14 is SEQ ID NO: 10; FC1 #15 is SEQ ID NO: 11; FC1 #16 is SEQ ID NO: 12; FH1 #1 is SEQ ID NO: 13; FH1 #3 is SEQ ID NO: 14; FH1 #10 is SEQ ID NO: 15; FH1 #20 is SEQ ID NO: 16; FH1 #22 is SEQ ID NO: 17; FH1 #24 is SEQ ID NO: 18; FH1 #41 is SEQ ID NO: 19; FH1 #42 is SEQ ID NO: 20; and FH1 #43 is SEQ ID NO: 21. In Figures 2E-2F the amino acid consensus sequence is SEQ ID NO: 22; FC1 #4 is SEQ ID NO: 23; FC1 #5 is SEQ ID NO: 24; FC1 #6 is SEQ ID NO: 25; FC1 #10 is SEQ ID NO: 26; FC1 #12 is SEQ ID NO: 27; FC1 #13 is SEQ ID NO: 28; FC1 #14 is SEQ ID NO: 29; FC1 #15 is SEQ ID NO: 30; FC1 #16 is SEQ ID NO: 31; FH1 #1 is SEQ ID NO: 32; FH1 #3 is SEQ ID NO: 33; FH1 #10 is SEQ ID NO: 34; FH1 #20 is SEQ ID NO: 35; FH1 #22 is SEQ ID

NO: 36; FH1 #24 is SEQ ID NO: 37; FH1 #41 is SEQ ID NO: 38; FH1 #42 is SEQ ID NO: 39; FH1 #43 is SEQ ID NO: 40.

**Figures 3A-3C** show HIV-1 Western blot analysis of subjects #FH1 and #FH2. Strongly reactive (++), weakly reactive (+), and non-reactive (-) control human sera from the Bio-Rad Novapath HIV-1 Immunoblot Kit and Cambridge Biotech HIV-1 Western Blot Kit were used as controls for respective HIV-1 Western blot strips. Serum from subject #FH5 with minimal exposure to cats, was used as additional negative control for Western blots from both companies. The durations of serum incubation are shown and FDA-approved recommended incubation periods are also designated with asterisk. Key bands are highlighted with an arrowhead on the left.

**Figure 4** shows gag nucleotide sequence comparison of cat #FC1, subject #FH1 and FIV strains. Gag sequences of cat #FC1 (SEQ ID NO: 42) and subject #FH1 were compared to all FIV strains available in our laboratory (SEQ ID NO: 43 is FIV<sub>PETALUMA</sub>; SEQ ID NO: 44 is FIV<sub>UK8</sub>; SEQ ID NO: 45 is FIV<sub>PPR</sub>; SEQ ID NO: 46 is FIV<sub>SENDAL-1</sub>; SEQ ID NO: 47 is FIV<sub>Bang</sub>; SEQ ID NO: 48 is FIV<sub>AOMORI-1</sub>; SEQ ID NO: 49 is FIV<sub>AOMORI-2</sub>; SEQ ID NO: 50 is FIV<sub>SENDAL-2</sub>; SEQ ID NO: 51 is FIV<sub>TM2</sub>; SEQ ID NO: 52 is FIV<sub>YOKOHAMA</sub>; SEQ ID NO: 53 is FIV<sub>SHIZUOKA</sub>; and SEQ ID NO: 54 is FIV<sub>FUKUOKA</sub>). The consensus sequence of subject #FH1 is shown at the top (SEQ ID NO: 41). Nucleotides identical to the consensus sequence of subject #FH1 (top sequence) are designated as a dot and those which differ from the consensus are presented with the appropriate nucleotide symbols. Gaps in sequence are presented as hyphens.

**Figures 5A-5E** show HIV-1 and FIV Western blot analysis of experimentally FIV-infected cats and pet cats. SPF cats #H3J, #D55, #455, and #X3D were experimentally infected with FIV<sub>Pet</sub> (subtype A), FIV<sub>UK8</sub> (subtype A), FIV<sub>Shi</sub> (subtype D), and FIV<sub>Bang</sub> (subtype A<sub>gag</sub>/B<sub>Env</sub>), respectively. FIV<sub>Bang</sub> has Gag sequence of FIV subtype A and Env sequence of FIV subtype B. These serum were reacted with HIV-1 Western blots (Figures 5A and 5B) or FIV Western blots (Figures 5C, 5D, and 5E). Serum samples of these cats before FIV infection were negative by both FIV and HIV-1 Western blot analyses (data not shown). Serum from pet cats #FC1 and #FC2 were also tested for their reactivity to HIV-1 and FIV antigens. Cat #C9V (7 months post-inoculation serum shown) is an SPF cat inoculated with FIV isolated from pet Cat #FC1. All sera were incubated at serum dilution of 1:100. All procedures are identical to those described in Figures 1 and 3 unless stated

otherwise. Key bands are highlighted with an arrowhead on the left. FDA-approved serum incubation periods of 20 hours for Cambridge Biotech HIV-1 Western Blot Kit (Figure 5A) and 0.5 hour for Bio-Rad Novapath HIV-1 Immunoblot Kit (Figure 5B) were performed with the cat sera. Serum incubation for FIV Western blots was 20 hours (Figures 5C, 5D, and 5E).

**Figures 6A-6C** show HIV-1 and HTLV-1/2 immunoblot analysis of FIV-infected and FIV-vaccinated cat sera. Sera from FIV-infected cats and FIV-vaccinated cats were tested for cross-reactive antibodies to HIV-1 with BioRad Novapath HIV-1<sub>UCD1</sub> and Cambridge Biotech HIV-1<sub>IIIb</sub> immunoblot kits (Figures 6A & 6B) and to HTLV-1/2 with Cambridge Biotech HTLV-1/2 immunoblot kit (Figure 6C). Selected cat sera with unique banding patterns are shown to demonstrate the presence of cross-reactive antibodies with various patterns of reactivity to HIV-1 proteins. Serum samples of these cats before FIV inoculation were negative by both HIV-1 and HTLV-1/2 immunoblot analyses (data not shown).

**Figures 7A and 7B** show temporal development of cross-reactive antibodies to HIV-1. FIV and HIV-1 immunoblots are shown using selected sera from: Figure 7A, FIV-infected cats from different weeks post-inoculation (wk pi or pi); and Figure 7B, FIV-vaccinated cats from different weeks post-vaccination (post-vaccination number). Sera were compared to their pre-inoculation or pre-vaccination sera (Pre).

**Figure 8** shows absorption of cat sera with viral antigens. Figure 8A: Cat sera were absorbed against inactivated FIV-infected cells followed by competition on HIV-1 immunoblots by inactivated FIV. Absorptions were also performed with PBS, uninfected cat FeT-J cells, and uninfected human H9/HuT-78 cells. Figure 8B: Sera were absorbed against PBS, uninfected cells lysate, or inactivated HIV-infected HuT-78 cells prior to incubation with HIV-1 immunoblot strips. Absorptions were performed for 2 hours at room temperature before development with anti-cat reagents. Figure 8C: FIV-vaccinated cat sera containing neutralizing antibodies to HIV-1 (Cat #C6G and #C9K) and sera from uninfected FeT-J cell immunized cats (Cats #C6E and #3G5) were tested at 1:100 dilution for reactivity to 5 µg/ml of either uninfected FeT-J cells, uninfected HuT-78 cells, or purified FIV<sub>Pet</sub>. Vaccinated cat sera had reactivity to FIV surface Env gp95 (arrow head). No significant reactivities were detected to uninfected FeT-J and HuT-78 proteins at 95 kDa, 120 kDa, and 160 kDa, suggesting that serum reactivity to HIV-1 and FIV envelopes were not due to

nonspecific reactivity to cellular proteins. In addition, cats immunized with uninfected FeT-J cells had no reactivity to cellular proteins at 95 kDa but had antibodies reactive to cellular proteins close to 120 kDa and 160 kDa. However, these anti-cellular antibodies were close but distinctly different from reactivity to HIV-1 gp120 and gp160. Figure 8D: Serum from a cat immunized with uninfected FeT-J cells was absorbed against PBS, FeT-J, H9/HuT-78 cells, and FIV-infected Fet-J cells. Reactivities in serum from Cat #305 were readily absorbed against uninfected cat and human cells. Immunoglobulin levels of all absorbed sera were not significantly altered by infected-cell absorptions when compared to PBS and uninfected-cell absorbed sera. Seven % PAGE gels were used for developing immunoblots to increase resolution of high molecular weight proteins. Molecular weights (M) are presented in kDa.

**Figures 9A and 9B** show reactivity of FIV-vaccinated cat sera and PBMC to HIV p24 and gp160. Figure 9A: Sera from cats immunized with dual-subtype FIV vaccine were tested by ELISA using recombinant HIV-1<sub>BRU</sub> p24, HIV-1<sub>IIIB</sub> gp160, and FIV p24. ELISA results at serum dilution of 1:300 are presented as mean difference between pre- and post-vaccination sera. Figure 9B: PBMC from dual-subtype FIV vaccinated cats at 2 weeks post-5th vaccination were tested for interferon- $\gamma$  production in response to recombinant HIV-1<sub>BRU</sub> p24, HIV-1<sub>IIIB</sub> gp160, and FIV p24. All PBMC stimulated with SEA were positive for IFN $\gamma$  production (data not shown). The average of the triplicate samples are shown for IFN $\gamma$  production. Standard deviations of the average IFN $\gamma$  titer were less than 10% of the mean.

**Figure 10** shows sequence alignments for partial FIV gag sequence from subject #FH1 PBMC following Real-time PCR. The Consensus sequence is SEQ ID NO: 55; SEQ ID NO: 56 is a partial FIV<sub>Pet</sub> gag sequence; SEQ ID NO: 57 is a partial FIV<sub>Bang</sub> sequence; SEQ ID NO: 58 is a partial FIV<sub>JSY3</sub> gag sequence; SEQ ID NO: 59 is a partial FIV<sub>UK8</sub> gag sequence; SEQ ID NO: 60 is a partial FIV<sub>Shizuoka</sub> sequence; SEQ ID NO: 61 is a partial FIV<sub>AOMORI 1</sub> sequence; SEQ ID NO: 62 is a partial FIV<sub>TM2</sub> gag sequence; SEQ ID NO: 63 is a partial FIV reverse transcriptase forward sequence; SEQ ID NO: 64 is a partial FIV reverse transcriptase probe sequence; SEQ ID NO: 65 is a partial FIV reverse transcriptase reverse sequence; SEQ ID NO: 66 is a FC1 gag sequence; SEQ ID NO: 67 is the A9=4 sequence; SEQ ID NO: 68 is the B4=5 sequence.

Please amend the Brief Description of the Sequences paragraph beginning on page 8, line 2:

**SEQ ID NO. 1** is a sense primer or amplification of FIV *gag* that can be used according to the present invention.

**SEQ ID NO. 2** is a antisense primer or amplification of FIV *gag* that can be used according to the present invention.

**SEQ ID NO: 3** is a nucleotide sequence of the present invention.

**SEQ ID NO: 4** is a nucleotide sequence of the present invention.

**SEQ ID NO: 5** is a nucleotide sequence of the present invention.

**SEQ ID NO: 6** is a nucleotide sequence of the present invention.

**SEQ ID NO: 7** is a nucleotide sequence of the present invention.

**SEQ ID NO: 8** is a nucleotide sequence of the present invention.

**SEQ ID NO: 9** is a nucleotide sequence of the present invention.

**SEQ ID NO: 10** is a nucleotide sequence of the present invention.

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**SEQ ID NO: 22** is an amino acid sequence of the present invention.

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